JOURNAL OF CHROMATOGRAPHY

PAPER ELECTROPHORESIS OF PROTEINS IN ACID BUFFER

Paper electrophoresis of proteins has been generally limited to pH values alkaline to the isoelectric points of proteins because positively charged proteins become adsorbed to paper[1]. However, some mixtures of protein are more separable by conventional electrophoresis in acid media than in alkaline media; such is the case for α-casein[2]. Although α-casein appears as a single component at pH 8.6 (veronal buffer), it exhibits two or more components at pH 2.3 using free-boundary electrophoresis[2]. These components differ in their sensitivity to precipitation by calcium ions. They have been referred to descriptively as calcium-sensitive α-casein and calcium-insensitive α-casein. Since paper electrophoresis has advantages over free-boundary electrophoresis for fractionation work, a suitable technique in the pH 2.3 region on paper was considered desirable.

Buffer materials for the pH 2.3 region are limited since many organic acids do not ionize sufficiently to provide buffering capacity there. Glycine, although adequate in this respect, produces turbidity in some casein solutions. Hydrochloric acid cannot be used in the Durrum type cell for paper electrophoresis because free chlorine is liberated at the anode. In addition to these limitations for acid electrophoresis of proteins, the limitation caused by the tendency of α-casein to travel as irregular broad bands at pH 8.6 (see Fig. 1) is also encountered in acid solution unless special steps are taken. In this study, these steps have been determined; applied to the α-casein complex and to its components, they permit the paper electrophoretic separation of bands corresponding to calcium-sensitive and calcium-insensitive α-caseins.

Paper electrophoresis was carried out in a Spinco Model R (Durrum type) cell. In each run, 8 strips of S & S 2043A filter paper were used; each strip measured 30.6 × 3.6 cm. The casein electropherograms were stained in accordance with procedures reported by LEVITON[4] for milk proteins.

The effect of various buffer materials on the adsorption of positively charged α-casein to filter paper was determined by a simple chromatographic procedure. If a spot of 1% casein was placed on a filter paper strip and chromatographed with an aqueous solution of buffer material, it could be seen that the spot (1) did not move with the solvent (adsorption), (2) moved as a streak from the origin to the solvent

* Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

front (partial adsorption), or (3) moved as a spot with the solvent front (no adsorption, and therefore ideal buffer material). When it was found that certain additives enhanced the buffer qualities, these materials were also used as chromatographic solvents even though they could not serve as buffer materials alone.

The following materials were screened by this chromatographic technique; glycolic acid; oxalic acid; lactic acid; acetic acid; propionic acid; galactose, sucrose, sodium potassium tartrate[5]; sodium octyl sulfate. Lactic acid was the only material which permitted minimal adsorption of the α-casein complex (one component was adsorbed) and provided adequate buffering at pH 2.3. When propionic acid was added to the lactic acid, no components of α-casein were adsorbed to the paper. The

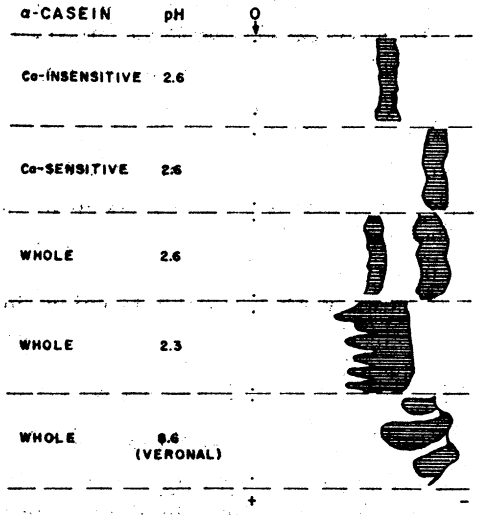

Fig. 1. Electrophoresis carried out 5 h, 250 V, 25°; buffer contains 50 ml lactic acid and 20 ml propionic acid per l, adjusted to pH values with 12.5 *N* NaOH; all samples are α-casein.

other materials tested were unsuitable. Several other α-hydroxyl acids besides lactic acid have sufficient dissociation values to permit buffering at pH 2.3 (α-hydroxybutyric, -valeric, and -caproic) but their expense or unpleasant odor will probably limit their use.

The results of the electrophoretic study of the caseins confirmed the results of chromatography; that is, a material conducive to a smeared chromatographic strip was also conducive to a smeared or blurred electrophoretic strip. The electrophoretic patterns using lactic acid alone or lactic acid plus propionic acid exhibited the same adsorption characteristics as the chromatographic patterns produced by them.

Even the buffer mixture (50 ml lactic acid (85 % purity) and 20 ml of propionic acid per liter, adjusted to pH 2.3) did not provide ideal paper electrophoretic patterns of the α-casein complex at 4°, since a densely stained component still moved as several broad streaks rather than a sharp band (Fig. 1). Electrophoresis at several pH values and several temperatures indicated that sharp bands are produced at pH 2.6 and 25°.

When 8 strips were used in the Durrum cell run at room temperature (25°), it was found that a 5 to 10 hour run at 250 V and 8 mA average was ideal. Overnight runs (16–17 h) at lower voltages (200 V) permitted too much diffusion to take place. Amperages as high as 16 mA caused such rapid evaporation that the apex of the paper strips became dry and further protein migration was halted. When the propionic acid content was increased from 20 ml to 22 ml/l, the bands produced were not as sharp as formerly.

Placing the samples at positions removed from the apex of the strips was of no advantage. The buffer flow—caused by evaporation from the strips and consequent replacement by reservoir buffer—was studied by placing lactose samples at various positions. It appeared that when electrophoretic migration was in the same direction as buffer flow, the bands were further apart, but more diffuse. When buffer flow was in the opposite direction, the bands were closer and sharper.

The slight "streaming" effect in the patterns did not alter the band positions. If this effect was produced by evaporation as suggested by SLOTTA *et al.*[6], it could be eliminated by horizontal electrophoresis between glass plates.

Samples of the α-casein complex, calcium-sensitive and calcium-insensitive α-caseins could be distinguished on paper electrophoresis with the conditions developed. These conditions consisted of the use of 50 ml of lactic acid, 20 ml of propionic acid, and 2 ml of 12.5 *N* NaOH per l giving pH 2.6 buffer and the running of samples in a Durrum cell with 8 strips for 5 h at 250 V, 8 mA (average) at 25°.

The α-casein complex exhibited bands at 4.0 cm and 6.0 cm. Calcium-insensitive α-casein produced one band at 4.3 cm and calcium-sensitive α-casein produced one band at 6.0 cm. The bands are illustrated in Fig. 1 along with patterns produced at pH 8.6 and pH 2.3 to demonstrate the improvement in resolution at pH 2.6

SUMMARY

An acid buffer solution (pH 2.3 to 2.6) has been devised, which permits paper electrophoresis of α-casein complex and its components. This buffer consists of lactic acid and propionic acid; it minimizes adsorption of protein to filter paper and produces sharp bands, which are characteristic of calcium-sensitive and calcium-insensitive α-caseins. Electrophoresis is carried out at 25° at 250 V for 5 hours.

REFERENCES

1 A. TISELIUS AND P. FLODIN, *Advances in Protein Chemistry*, Vol. 8, Academic Press, Inc., New York, 1953, p. 471.
2 T. L. MCMEEKIN, N. J. HIPP AND M. L. GROVES, *Arch. Biochem. Biophys.*, (in the press).
3 D. F. WAUGH AND P. H. VON HIPPEL, *J. Am. Chem. Soc.*, 78 (1956) 4576.
4 A. LEVITON, *Agricultural and Food Chemistry*, 5, No. 7 (1957) 532.
5 A. E. FRANKLIN, J. H. QUASTEL AND S. F. VAN STRATEN, *Proc. Soc. Exptl. Biol. Med.*, 77 (1951) 783.
6 K. SLOTTA, S. BRIL AND A. BALLESTER, *Z. physiol. Chem.*, 296 (1954) 141.